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Activation of Dynamin I Gene Expression by Sp1 and Sp3 Is Required for Neuronal Differentiation of N1E-115 Cells*

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Dynamin I is a key molecule required for the recycling of synaptic vesicles in neurons, and it has been known that dynamin I gene expression is induced during neuronal differentiation. Our previous studies established that neuronal restriction of dynamin I gene expression is controlled by Sp1 and nuclear factor-κB-like element-1. Here, using a series of deletion constructs and site-directed mutation, we found that transcription of dynamin I gene during neuronal differentiation of N1E-115 cells is controlled primarily by the Sp1 element located between −13 to −4 bp of the dynamin I promoter. Gel shift analysis demonstrated that in addition to Sp1, Sp3 could interact with this Sp1 element. The requirement for Sp family transcription factors in dynamin I gene expression was confirmed by using mithramycin, an inhibitor of Sp1/Sp3 binding. Mithramycin repressed dynamin I gene expression and resulted in blocking of neuronal differentiation of N1E-115 cells. The localization of the dynamin I protein was also restricted in the peripheral region of the nucleus by the mithramycin treatment. Thus, all of our results suggest that induction of dynamin I gene expression during N1E-115 cell differentiation is modulated by Sp1/Sp3 interactions with the dynamin I promoter, and its expression is important for neuronal differentiation of the N1E-115 cells.

Dynamin is a GTPase protein that is believed to play a key role in detaching clathrin-coated vesicles from the plasma membrane (1, 2). The role of dynamin in receptor-mediated endocytosis in mammalian cells has been confirmed both *in vivo* and *in vitro* (3–6), but its exact function remains controversial (7, 8). In brain, three different isoforms of dynamin (brain-specific dynamin I (9, 10), ubiquitously expressed dynamin II (11), and dynamin III that was originally described as being testis-specific (12)) are expressed simultaneously. It has been known that the mRNA levels of dynamin I and III are up-regulated throughout brain development, whereas the levels of dynamin II mRNA remain unchanged (12, 13). Therefore, understanding the regulation mechanism that confers cell type- and development stage-specific expression of dynamin I is important to know the *in vivo* role of this protein. To understand the molecular mechanism of tissue-specific dynamin I gene expression, we previously cloned and analyzed the 5′-flanking regions of the mouse dynamin I gene and reported that Sp1, NF-κB-like element (NE)[1]-1, and neuron restrictive silencer element are required for the promoter activity of dynamin I gene in neuronal cells (14, 15). We also reported that YY1 binds to the negative regulatory region of the dynamin I gene promoter and strongly represses dynamin I promoter activity (16).

The N1E-115 neuroblastoma cells are capable of forming neurites when grown in the absence of serum or in the presence of Me_2SO (17–19). Previously, Torre *et al.* reported that dynamin I levels increase steadily with the formation of neurites and decrease during the serum-induced neurite retraction in the N1E-115 cells (20). Furthermore, a reduction in the intracellular level of dynamin in the hippocampal neurons through antisense oligonucleotide treatment results in a significant impairment in neurite formation. Therefore, these results provided convincing evidence demonstrating that dynamin I is required for normal neuritogenesis.

To study the molecular mechanism of the up-regulation of dynamin I gene expression during the differentiation of N1E-115 cells, we analyzed the promoter activity of the dynamin I gene in this cell. Deletion analysis in conjunction with site-directed mutagenesis showed that an Sp1 site at −14 to −7 is critical for Me_2SO-induced dynamin I promoter activity. Gel shift analysis demonstrated that in addition to Sp1, Sp3 could interact with this Sp1 element. We also confirmed that Sp family transcription factors are functionally important since mithramycin, an inhibitor of Sp1/Sp3 binding (21), represses dynamin I gene expression. Furthermore, a reduction in the intracellular level of dynamin I in N1E-115 cells through mithramycin treatment resulted in a significant impairment in neurite formation. These results provide evidence that the dynamin I gene is transcriptionally activated during Me_2SO-induced neuronal differentiation of N1E-115 cells through an Sp1 element, and this activation is essential for neuronal differentiation of the N1E-115 cells.

EXPERIMENTAL PROCEDURES

Reporter Plasmid Construction and Transient Transfection Procedure—All of the deletion and mutant constructs of the dynamin I promoter used in this study were previously described (14).

Cell Culture and Transient Transfection Assay—N1E-115 cells were passaged in Dulbecco's modified Eagle's medium containing 4.5 g of glucose/liter and supplemented with 10% fetal bovine serum and 2 mM glutamine. All culture media contained 100 units of penicillin and 100

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[1] The abbreviations used are: NE, NF-κB-like element; CAT, chloramphenicol acetyltransferase; PBS, phosphate-buffered saline; EMSA, electrophoretic mobility shift assay; Ab, antibody.

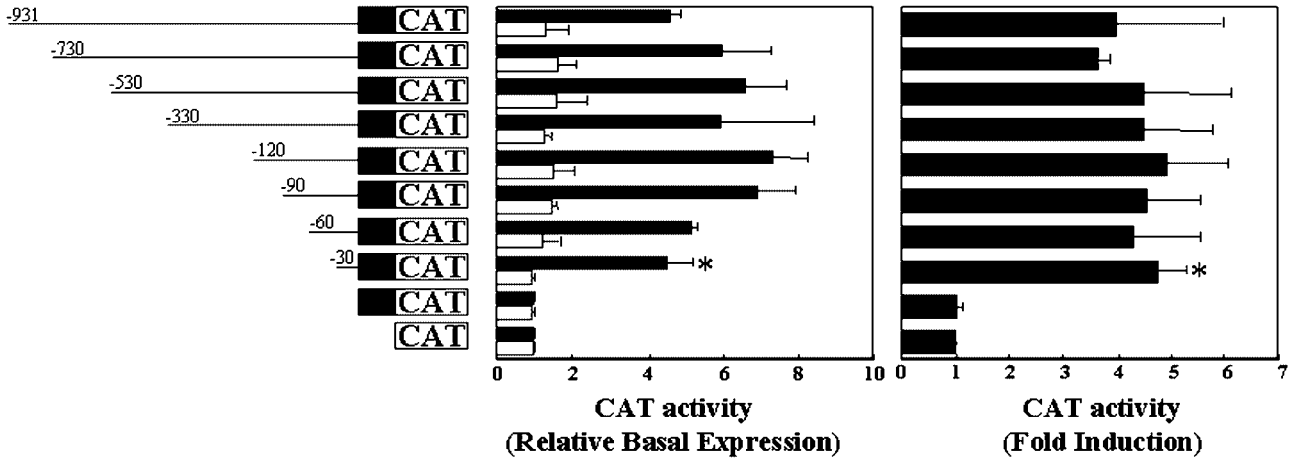

FIG. 1. **Characterization of the Me_2SO-inducible regions of the dynamin I promoter in N1E-115 cells.** A series of deletion constructs (*left*) were cotransfected with a pCMV-β standardization plasmid into N1E-115 cells, and the cells were either grown under control condition (*middle*, *white bars*) or in the presence of 1.5% Me_2SO (*middle*, *black bars*) for 48 h. The cells were harvested, extracts were prepared, CAT and β-galactosidase activities were determined, and β-galactosidase activity was used to correct for variations in transfection efficiency. The promoter activities of each dynamin I-CAT fusion constructs were determined and expressed as a fold of CAT activity of the pCAT-Basic. Fold of induction was calculated by dividing the corrected induced value by the corrected control value (*right*). The results, expressed as mean ± S.E., represent data obtained in three independent experiments. *Asterisks* indicate statistically significant induction in the presence of 1.5% Me_2SO as determined by Student's *t* test ($p < 0.05$).

μg/ml streptomycin. Cells were subcultured from confluent dishes by diluting the cells 1:4 with fresh medium 3 days prior to transfection. All of the dynamin I promoter constructs were transfected into N1E-115 cells by means of FuGENE 6 (Roche Molecular Biochemicals). The cells were allowed to adhere to the dish for 12 h; 1.5% Me_2SO was added to one dish of each pair, and the cells were incubated for an additional 48 h.

CAT and β-Galactosidase Assays—To prepare cell extracts for CAT and β-galactosidase expression assay, cells were scraped and centrifuged at 4 °C for 5 min. The cell pellet was resuspended in 150 μl of 0.25 M Tris, pH 7.8, and then subjected to three freeze-thaw cycles. Cell debris was centrifuged at 4 °C for 5 min. The resulting supernatant was removed and used directly in the assays. β-Galactosidase assay was carried out using a chlorophenol red-β-D-galactopyranoside (Roche Molecular Biochemicals) and used to normalize CAT activities. β-Galactosidase activity was measured using 10 μl of cell extract in a reaction mixture consisting of 2.5 mM chlorophenol red-β-D-galactopyranoside and 1.25 mM $MgCl_2$. After incubation for 0.5–1 h at 37 °C, the reaction was stopped by the addition of 3 mM $ZnCl_2$, and the absorbance of reaction product was read at 574 nm. CAT activity was determined using 50–100 μl of cell extract in the presence of acetyl-CoA and [^{14}C]chloramphenicol in a total volume of 150 μl. The mixture was incubated at 37 °C for 2 h, extracted with ethyl acetate, and dried in a vacuum desiccator. Acetylated and non-acetylated forms of [^{14}C]chloramphenicol were resuspended in 20 μl of ethyl acetate and separated by thin-layer chromatography for 30 min at room temperature with chloroform/methanol (97:3, v/v) as the mobile phase. Percentage conversion of chloramphenicol to its acetylated forms was determined using phosphorimaging.

Reverse Transcription-PCR—Total RNA was prepared from N1E-115 cells grown either in the presence or absence of Me_2SO. Mithramycin (200 nM) was cotreated with Me_2SO for 48 h. Five micrograms of total RNA were mixed with 300 ng of oligo(dT) primer and heated at 65 °C for 5 min, cooled slowly at room temperature, and then reverse transcribed using StrataScript reverse transcriptase (Stratagene) at 42 °C for 1 h as recommended by the supplier. One-tenth of the reverse-transcribed mixture was PCR-amplified using forward primer 5′-TCTGAAGCTGCGTGATGTG-3′ (+1694 to +1713) and reverse primer 5′-CATCGAGTGCATGAAGCTGT-3′ (+1941 to +1922) relative to the first ATG in the coding sequence of the dynamin I gene. Thirty cycles of PCR were done at 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min.

Preparation of Nuclear Extracts and Electrophoretic Mobility Shift Assay—N1E-115 cells were propagated as described for transfection analysis and treated with 1.5% Me_2SO or grown under control conditions in 15-cm dishes. The cells were washed with ice-cold phosphate-buffered saline (PBS) and resuspended in 1 ml of lysis buffer (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$). The cells were incubated on ice for 15 min, and the nuclei were then pelleted by centrifugation for 15 min at 3000 × *g* at 4 °C. The pellet was resuspended in 200 μl of extraction buffer (30 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 0.2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, leupeptin, 500 mM KCl, 10% glycerol). The cells were incubated on ice for 30 min and pelleted by centrifugation for 20 min at 12,000 × *g* at 4 °C. The supernatant was recovered as the nuclear extract and protein concentrations were determined by a modified Bradford method (Bio-Rad). For electrophoretic mobility shift assay, 8 μg of nuclear extract proteins were mixed with the binding buffer (30 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 0.2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin,leupeptin, 100 mM KCl, 10% glycerol, 0.5 μg of poly(dI-dC)). Double stranded ^{32}P-labeled oligonucleotides containing an Sp1 sequence were used as a probe. In the competition experiments, a 100-fold molar excess of unlabeled oligomers (wild and mutated Sp1 oligonucleotides) were incubated with the nuclear extracts before addition of ^{32}P-labeled oligonucleotides. For supershift analysis, 1 μl of antibody specific for Sp1, Sp2, Sp3, or Sp4 (Santa Cruz Biotechnology) was preincubated with nuclear extract proteins for 1 h at 4 °C before addition of the DNA probe. To block Sp1 binding to DNA, DNA probes were preincubated for 1 h at 4 °C with mithramycin (200 nM) before being used in binding reactions. Protein/DNA complexes were fractionated by electrophoresis in nondenaturing 5% polyacrylamide gels for normal EMSA experiments or in nondenaturing 4% polyacrylamide gels for supershift analysis and visualized by autoradiography.

Light Microscopy, Confocal Laser Scanning Microscopy, and Data Analysis—The formation of neurites in N1E-115 cells by Me_2SO treatment and blocking of neurites formation by different concentration of mithramycin (100–300 nM) treatment were monitored by inverted microscopy (Axiovert 25 microscope, Zeiss). Pictures were taken after 48 h of each treatment. For immunofluorescence assay, N1E-115 cells treated with different concentration of mithramycin were grown on glass coverslips for 48 h to 60% confluence. The coverslips were rinsed briefly in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO4, 1.4 mM KH_2PO4, pH 7.4) and fixed for 20 min at room temperature in 4% paraformaldehyde fixative, followed by permeabilization with 0.1% Triton X-100. The cells were blocked with 1.0% bovine serum albumin in PBS and incubated for 1 h at room temperature with monoclonal antibody against dynamin I (BD Transduction Laboratories) as primary antibody diluted in PBS containing 1.0% bovine serum albumin. After three washes in PBS, the coverslips were incubated with affinity-isolated secondary antibody (fluorescein-conjugated goat anti-mouse IgG) (BIOSOURCE, Camarillo, CA). The coverslips were washed three times with PBS and mounted in fluorescent mounting medium (DAKO). Cells were observed for epifluorescence using a confocal laser scanning microscope (TCS400, LEICA, Inc.). Acquired images were manipulated with SCANware 5.0 (LEICA, Inc.) and digitized using Adobe PhotoShop Software (Adobe Photosystems, Inc., Mountain View, CA). All data are depicted as mean ± S.E. Differences between two groups were validated by Student's *t* test.

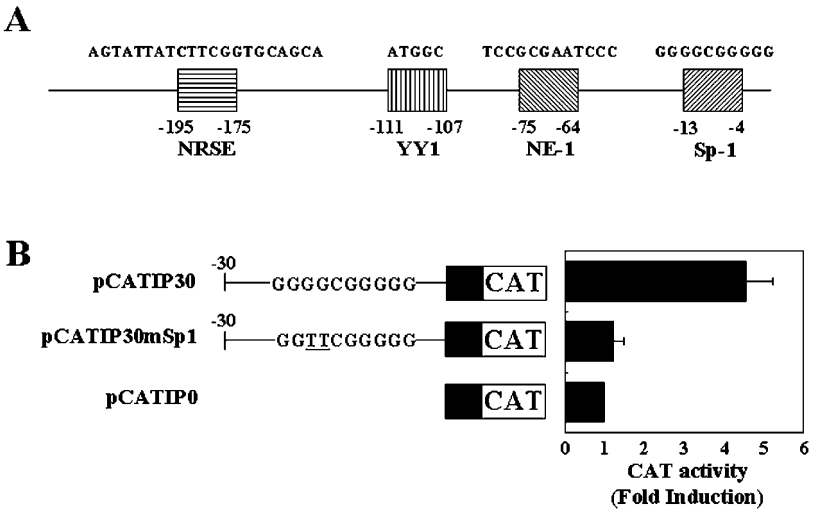

FIG. 2. **Functional role of the Sp1 element in dynamin I promoter and analysis by mutagenesis.** *A*, schematic diagram of the mouse dynamin I promoter. Important cis-acting elements of dynamin I promoter, which we previously published, were shown. *B*, N1E-115 cells transiently transfected with wild type (pCATIP30), Sp1 mutant (pCATIP30mSp1), and control vector were treated with 1.5% Me_2SO for 48 h prior to lysis for CAT activity assays. β-Galactosidase activity was used to correct for variations in transfection efficiency. The results, expressed as mean ± S.E., represent data obtained in three independent experiments.

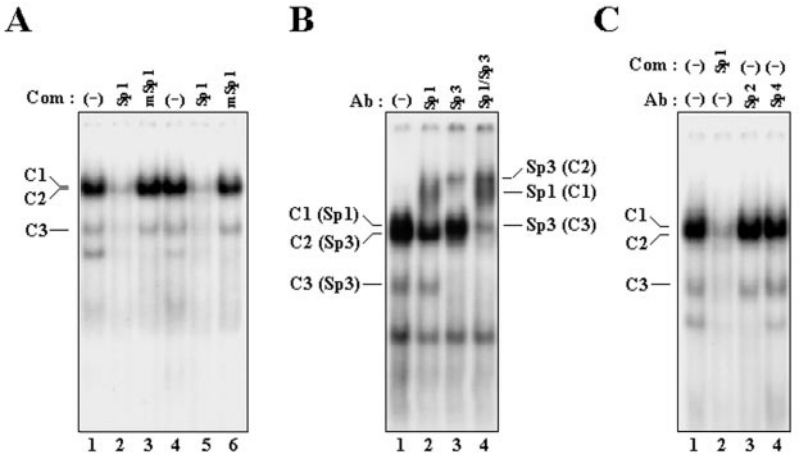

FIG. 3. **Sp1 and Sp3 bind to the dynamin I promoter.** *A*, EMSA was performed with 8 μg of nuclear extracts from N1E-115 cells that had either been treated with 1.5% Me_2SO (*lanes 4–6*) for 48 h or grown under control conditions (*lanes 1–3*). When the labeled Sp1 oligonucleotide was used, three specific DNA/protein complexes (*C1–C3*) were formed (*lanes 1* and *4*). Competition analysis was performed in the presence of a 100-fold molar excess of unlabeled Sp1 (*lanes 2* and *5*) and mSp1 (*lanes 3* and *6*) oligonucleotide. *B*, binding of Sp1 and Sp3 to probe Sp1 was analyzed by supershift assay using anti-Sp1 and anti-Sp3 Abs. A nuclear extract form N1E-115 cells that had been treated with 1.5% Me_2SO for 48 h was incubated with indicated Abs and then mixed with ^{32}P-labeled Sp1 probe. Complexes (*C1–C3*) with Sp1 or Sp3 and further shifted bands with anti-Sp1 (*lanes 2* and *4*) and anti-Sp3 (*lanes 3* and *4*) Abs are indicated. *C*, supershift analysis was performed in the presence of anti-Sp2 (*lane 3*) and anti-Sp4 (*lane 4*) Abs.

RESULTS

Cis-acting Elements for Me_2SO-induced Dynamin I Promoter Activity in N1E-115 Neuroblastoma Cells—It has been known that the dynamin levels increase during neurite formation in N1E-115 cells. To examine whether the activity of the mouse dynamin I promoter we previously cloned is activated during neurite formation, pCATIP931 containing a 931-bp 5′-flanking sequence of the mouse dynamin I gene (−931-+105) fused to the promoterless CAT gene in pCAT-Basic was transfected in N1E-115 cells, and the cells were either grown under control conditions or treated with 1.5% Me_2SO for 48 h. To control for variations in transfection efficiency, pCMVβ plasmid was co-transfected, and β-galactosidase activity was used to standardize CAT activity. As seen in Fig. 1, Me_2SO treatment resulted in a 4-fold increase in dynamin I promoter activity.

To determine the region that is responsible for this induction, progressive 5′ deletion mutants of the dynamin I promoter were constructed, and their promoter activities were assayed in N1E-115 cells (Fig. 1). The response to Me_2SO was maintained through deletion to −31. However, extension of the 5′ deletion to nt −1 drastically abrogated the response. These results indicate that the Me_2SO regulatory region lies in the 30-bp region located between −30 and −1.

Dynamin I Promoter Activity Is Regulated by the Transcription Factors Sp1 and Sp3—To elucidate the molecular mechanism by which expression of dynamin I is tissue specifically regulated, we previously cloned and characterized the promoter of mouse dynamin gene and reported that Sp1, NE-1, and neuron-restrictive silencer element are required for the promoter activity of dynamin I gene in neuronal cells (14, 15). We also reported that YY1 has a negative role in regulation of the dynamin I gene promoter activity (16). One Sp1 DNA binding site is located in this Me_2SO regulatory region (Fig. 2*A*). To define whether this element contributes to the response to Me_2SO, the Sp1 binding site was mutated, and the effect of this mutation on promoter activity was determined. Mutation of the Sp1 binding site in pCATIP30 nearly abrogated the response to Me_2SO (Fig. 2*B*). This result suggests that the Sp1 binding site is critical for the Me_2SO-induced dynamin I promoter activity.

To examine protein binding to this element, we used electrophoretic mobility shift assays with a radiolabeled Sp1 element located in the Me_2SO regulatory region as a probe and extracts from N1E-115 cells either grown under control conditions or treated with 1.5% Me_2SO for 48 h. We found that this probe specifically binds to protein complexes designated as C1 to C3

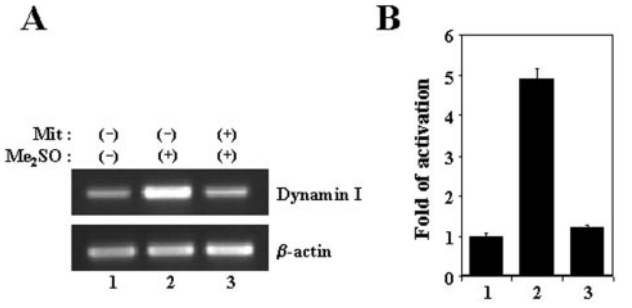

FIG. 4. **Mithramycin blocks the Me_2SO-induced expression of the dynamin I gene.** *A*, N1E-115 cells were treated with Me_2SO in the presence (*lane 3*) or absence (*lane 2*) of mithramycin for 48 h. Total RNA was isolated and analyzed for dynamin I and β-actin mRNA levels by reverse transcription-PCR. The location of the dynamin I and β-actin bands is shown. *B*, quantitative scanning densitometry of dynamin I band relative to β-actin bands from 4*A*. Each data point represents the mean ± S.E., and data was obtained in three independent experiments.

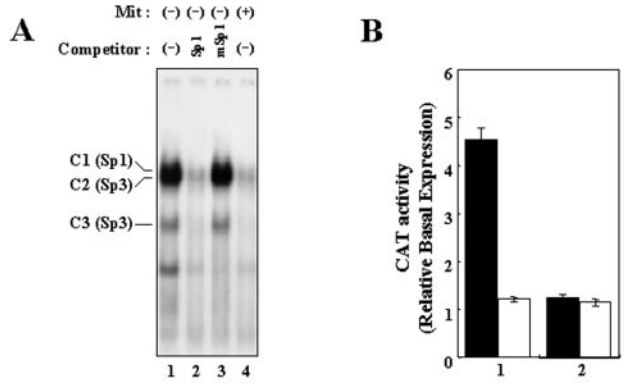

FIG. 5. **Mithramycin blocks Sp1/Sp3 binding and dynamin I promoter activity.** *A*, Sp1 oligonucleotide, spanning nt −13 to −4, was preincubated in the presence of mithramycin (200 nM, *lane 4*) at 4 °C for 1 h and subsequently used in gel shift analysis with nuclear extracts from N1E-115 cells that had been treated with 1.5% Me_2SO for 48 h. Complexes (*C1–C3*) with Sp1 or Sp3 are indicated. *B*, effect of mithramycin on dynamin I promoter activity. N1E-115 cells were transfected with either pCATIP30 (*lane 1*) or pCATIP30mSp1 (*lane 2*) construct and treated with Me_2SO in the presence (*white bars*) or absence (*black bars*) of mithramycin for 48 h. The cells were harvested, extracts were prepared, CAT and β-galactosidase activities were determined, and β-galactosidase activity was used to correct for variations in transfection efficiency. Each data point represents the mean ± S.E., and data was obtained in three independent experiments.

from N1E-115 cells either grown under control conditions or treated with 1.5% Me_2SO (Fig. 3*A*). Complex formation was inhibited specifically by adding excess amounts of unlabeled Sp1 consensus oligonucleotide (*lanes 2* and *5*) but not by the Sp1 mutant oligonucleotide (*lane 3* and *6*). To confirm that Sp1 binds to this element, we performed EMSA in the presence of Sp family antibodies to demonstrate supershifting. An oligonucleotide containing the Sp1 element located in the Me_2SO regulatory region was incubated with nuclear extracts from N1E-115 cells treated with 1.5% Me_2SO and Sp1- and Sp3-specific antibodies (Fig. 3*B*). Supershift assays demonstrated that complex C1 disappeared with an anti-Sp1 Ab (*lanes 2* and *4*), revealing a further shifted complex (*lanes 2* and *4*, *Sp1 (C1)*). Complexes C2 and C3 also disappeared with the anti-Sp3 Ab (*lanes 3* and *4*) with a new shifted complex appearing (*lanes 3* and *4*, *Sp3 (C2)*). Two complexes disappeared with the anti-Sp3 Ab, suggesting that this Ab binds to two different sized Sp3 as described previously (22). However, only one further shifted band was observed (*lane 3*, *Sp3 (C2)*). It is conceivable that a further shifted band is located, and thus not detected, in the same position as complex C1. Indeed, a weak band was observed at the same position as C1 with anti-Sp1 and -Sp3 Abs (*lane 4*, *Sp3 (C3)*). Incubation of the binding reactions with either anti-Sp2 or anti-Sp4 antibodies did not affect the EMSA pattern (Fig. 3*C*). All of these results suggested that Sp1 and Sp3 bind to the Sp1 element spanning −13 to −4, and this element is critical for the Me_2SO-induced dynamin I promoter activity.

Mithramycin Treatment Reduces Dynamin I Levels and Prevents Neurite Formation—To further investigate the role of Sp family on the regulation of dynamin I expression, we analyzed the effects of mithramycin, a drug that modifies GC-rich regions of the DNA and blocks Sp1 binding (21), on the expression of dynamin I in N1E-115 cells induced by Me_2SO. As shown in Fig. 4, Me_2SO induced dynamin I gene expression about 5-fold (Fig. 4, *A*, *B*, *lane 2*) but cotreatment of mithramycin with Me_2SO completely abolished the accumulation of dynamin I mRNA in Me_2SO-induced N1E-115 cells (Fig. 4, *A*, *B*, *lane 3*), which strongly suggests that Sp1 binding is essential for dynamin I expression. In gel shift experiments performed in the presence or absence of mithramycin, we confirmed that the drug inhibited Sp1 and Sp3 binding to the Sp1 element spanning −13 to −4 (Fig. 5*A*). To confirm whether dynamin I promoter activity is also repressed by treatment of mithramycin, we determined dynamin I promoter activity in the presence of mithramycin (Fig. 5*B*). Treatment of mithramycin with Me_2SO completely reduced pCATIP30 activity but not pCATIP30mSp1 activity. These results clearly suggest that

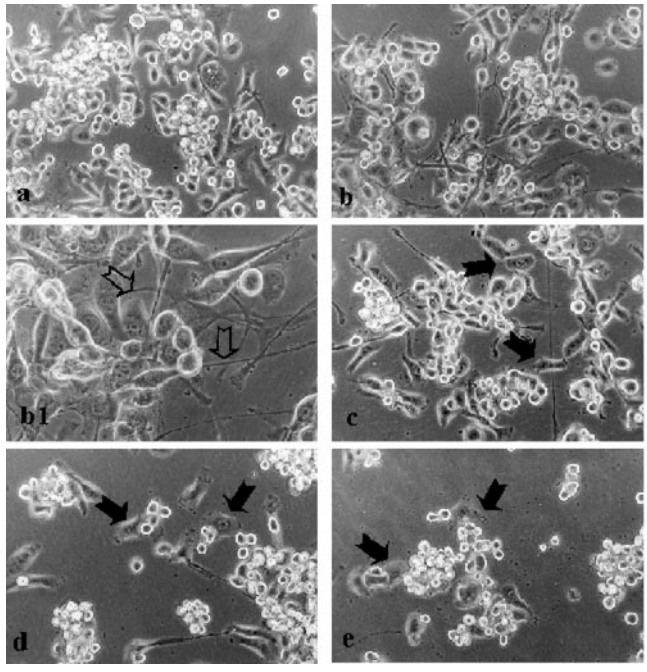

FIG. 6. **Treatment of mithramycin prevents neurite formation during differentiation of N1E-115 cells.** N1E-115 cells were treated with Me_2SO in the presence (*c*, 100 nM; *d*, 200 nM; *e*, 300 nM) or absence (*b* and *b1*) of mithramycin for 48 h. *a*, cells were grown under control condition. Mithramycin inhibits formation of neurites and cell growth with a dose-dependent manner (*closed arrows*). *Open arrows* indicate neurites. Magnifications of all images are X200 except b1 (X400).

mithramycin abolished dynamin I gene expression by repressing the binding between Sp family transcription factors and the Sp1 element in dynamin I promoter.

It has been known that normal levels of dynamin I are necessary for the formation of neurites in cultured hippocampal neurons. Therefore, we reduced the intracellular level of dynamin I in N1E-115 cells by mithramycin treatment and measured the effect on neurite outgrowth. As shown in Fig. 6, the morphology of N1E-115 cells was altered dramatically in

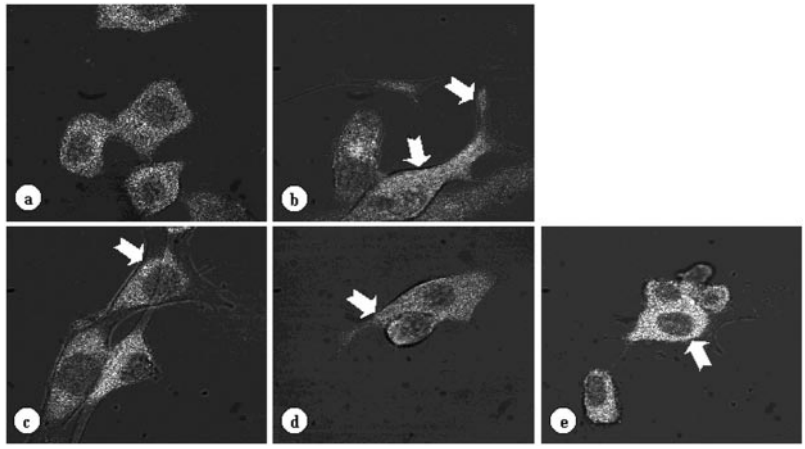

FIG. 7. **Localization of dynamin I protein was changed by treatment of mithramycin.** In differentiated cells dynamin I proteins are dispersed in cytoplasm and cell processes including neurites (*b*, *closed arrows*); however, in mithramycin-treated cells (*c*, 100 nM; *d*, 200 nM; *e*, 300 nM) their distribution was restricted in the peripheral region of the nucleus (*c*, *d*, *e*, *closed arrows*). *a*, cells were grown under control condition.

the presence of increasing concentrations of mithramycin. In the mithramycin-treated cells, a significant reduction in the number and length of neurites was observed (Fig. 6, *c–e*). This inhibitory effect on neurite outgrowth was dose-dependent. We also observed that the localization of dynamin is restricted in peripheral region of nuclear in mithramycin-treated cells (Fig. 7, *c–e*), whereas dynamin I proteins are dispersed in cytoplasm and cell processes including neurites in differentiated cells (Fig. 7*b*). All of these results definitely suggest that induction of dynamin I gene expression and exact localization are critical for neurite formation.

DISCUSSION

It has been known that dynamin I mRNA and protein levels increase during differentiation of N1E-115 cells and that normal levels of dynamin I are necessary for the formation of neurites (20). In this study we found that an Sp1 element located between −13 to −4 bp of the dynamin I promoter is responsible for a major part of dynamin I promoter activity during differentiation of N1E-115 cells, and Sp3 as well as Sp1 bound to this element. We also confirmed the importance of the Sp1 element by using mithramycin, a drug that modifies GC-rich regions of the DNA and blocks Sp1/Sp3 binding. Treatment of mithramycin inhibited Sp1 and Sp3 binding to the Sp1 element and completely abolished the accumulation of dynamin I mRNA and promoter activity in Me_2SO-induced N1E-115 cells, which strongly suggests that Sp1/Sp3 binding is essential for dynamin I expression. Blocking of dynamin I gene expression by mithramycin treatment resulted in a significant reduction of neurite formation. The localization of dynamin also changed from cytoplasm and cell processes including neurites to peripheral region of nuclear. All of these results clearly suggest that induction of dynamin I gene expression and exact localization are critical for neurite formation.

Dynamin is a GTPase that plays a critical role in endocytosis. Although the majority of studies implicate dynamin in endocytosis, there are many evidences to suggest that dynamin may play additional functions in cell physiology. Recently, many observations also link dynamin to the actin cytoskeleton. When the dynamin K44A mutant is overexpressed, the distribution of actin stress fibers and cell shape are altered (5, 23). Dynamin was shown to colocalize with filamentous actin at membrane ruffles (24) and also detected at podosomes (25). They also suggested that a direct functional link of dynamin to the actin cytoskeleton exists (25). All of these results and our results are convergent with those of recent studies reporting that dynamin directly binds regulatory components of the actin cytoskeleton, such as profilin (26), proteins of the syndapin/pacsin/FAP52 family (27–29), and cortactin (30).

Sp1 is a well characterized sequence-specific DNA-binding protein that is important for transcription of many cellular and viral genes that contain GC boxes in their promoters (31, 32). Three Sp1-related transcription factors (Sp2, Sp3, and Sp4) have been cloned. Sp2 does not recognize the same sequence as Sp1, and Sp4 expression is restricted to the brain. Sp3, on the other hand, is ubiquitously expressed and recognizes the same sequence as Sp1. Although Sp1 appears to be almost exclusively an activating transcription factor, Sp3 contains a transcriptional repression domain and can act as a transcriptional activator or repressor depending on the promoter and cell type studied (33, 34). While Sp1 and Sp3 are ubiquitous nuclear factors, the differences in the level of expression during different stages of development (35, 36) or in varying cell types (36) along with specific posttranslational modifications (37) are responsible for altering gene transcription in a development-specific and cell-specific manner. In addition, despite its general role in transcription of housekeeping genes, Sp1 has been demonstrated to be involved in induced transcription of various genes responding to different biological stimuli (38–40). However, total amounts of Sp1 and Sp3 proteins were not changed regardless of the differentiation state of N1E-115 cells, and they bound to the Sp1 element spanning −13 to −4 not only in Me_2SO-induced cells but also in control cells (Fig. 3*A*). It is still possible that other Me_2SO-inducible transcription factors weakly bind to this element and enhance the promoter activity. Another possibility is that binding of some Me_2SO-inducible transcription factors to their binding sites is dependent on the interaction with Sp1 or Sp3. It has been reported that transient interaction of Purα and Sp1 may result in stable association of Purα to its binding element, and these factors synergistically stimulate MBP promoter activity in central nervous system cells (41). Therefore, further studies involving identification of proteins that bind with Sp1 or Sp3 will facilitate elucidation of the dynamin I gene expression.

REFERENCES

1. Schmid, S. L. (1997) *Annu. Rev. Biochem.* **66,** 511–548
2. Schmid, S. L., McNiven, M. A., and De Camilli, P. (1998) *Curr. Opin. Cell Biol.* **10,** 504–512
3. Herskovits, J. S., Burgess, C. C., Obar, R. A., and Vallee, R. B. (1993) *J. Cell Biol.* **122,** 565–578
4. van der Bliek, A. M., Redelmeier, T. E., Damke, H., Tisdale, E. J., Meyerowitz, E. M., and Schmid, S. L. (1993) *J. Cell Biol.* **122,** 553–563
5. Damke, H., Baba, T., Warnock, D. E., and Schmid, S. L. (1994) *J. Cell Biol.* **127,** 915–934
6. Simpson, F., Hussain, N. K., Qualmann, B., Kelly, R. B., Kay, B. K., McPherson, P. S., and Schmid, S. L. (1999) *Nature Cell Biol.* **1,** 119–124
7. Sever, S., Muhlberg, A. B., and Schmid, S. L. (1999) *Nature* **398,** 481–486
8. Sever, S., Damke, H., Schmid, S. L. (2000) *Traffic* **1,** 385–392
9. Shpetner, H. S., and Vallee, R. B. (1989) *Cell* **59,** 421–432
10. Kosaka, T., and Ikeda, K. (1983) *J. Neurobiol.* **14,** 207–225
11. Cook. T. A., Urrutia, R., and McNiven, M. A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 644–648
12. Cook, T., Mesa, K., and Urrutia, R. (1996) *J. Neurochem.* **67,** 927–931
13. Faire, K., Trent, F., Tepper, J. M., and Bonder, E. M. (1992) *Proc. Natl. Acad.*

Sci. U. S. A. **89,** 8376–8380
14. Yoo, J., Lee, S. S., Jeong, M. J., Lee, K. I., Kwon, B. M., Kim, S. H., Park, Y. M., and Han, M. Y. (2000) *Biochem. J.* **351,** 661–668
15. Yoo, J., Jeong, M. J., Lee, S. S., Lee, K. I., Kwon, B. M., Kim, D. S., Park, Y. M., and Han, M. Y. (2001) *Biochem. Biophys. Res. Commun.* **283,** 928–932
16. Yoo, J., Jeong, M. J., Lee, S. S., Lee, K. I., Kwon, B. M., Park, Y. M., and Han, M. Y. (2001) *Biochem. Biophys. Res. Commun.* **283,** 340–343
17. Prasad, K. N. (1975) *Biol. Rev. Camb. Philos. Soc.* **50,** 129–165
18. Jalink, K., Eichholtz, T., Postma, F. R., van Corven, E. J., and Moolenaar, W. H. (1993) *Cell Growth Differ.* **4,** 247–255
19. Kimhi, Y., Palfrey, C., Spector, I., Barak, Y., and Littauer, U. Z. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73,** 462–466
20. Torre, E., McNiven, M. A., and Urrutia, R. (1994) *J. Biol. Chem.* **269,** 32411–32417
21. Greenwell, P., Inagaki, Y., Hu, W., Walsh, M., and Ramirez, F. (1997) *J. Biol. Chem.* **272,** 19738–19745
22. Hagen, G., Müller, S., Beato, M., and Suske, G. (1994) *EMBO J.* **13,** 3843–3851
23. Altankov, G., and Grinnell, F. (1993) *J. Cell Biol.* **120,** 1449–1459
24. Cao, H., Garcia, F., and McNiven, M. A. (1998) *Mol. Biol. Cell* **9,** 2595–2609
25. Ochoa, G.-C., Slepnev, V. I., Neff, L., Ringstad, N., Takei, K., Daniell, L., Cao, H., McNiven, M., Baron, R., and DeCamilli, P. (2000) *J. Cell Biol.* **150,** 377–390
26. Witke, W., Podtelejnikov, A. V., Di Nardo, A., Sutherland, J. D., Gurniak, C. B., Dotti, C., and Mann, M. (1998) *EMBO J.* **17,** 967–976
27. Merilainen, J., Lehto, V. P., and Wasenius, V. M. (1997) *J. Biol. Chem.* **272,** 23278–23284
28. Qualmann, B., Roos, J., DiGregorio, P. J., and Kelly, R. B. (1999) *Mol. Biol. Cell* **10,** 501–513
29. Ritter, B., Modregger, J., Paulsson, M., and Plomann, M. (1999) *FEBS Lett.* **454,** 356–362
30. McNiven, M. A., Kim, L., Krueger, E. W., Orth, J. D., Cao, H., and Wong, T. W. (2000) *J. Cell Biol.* **151,** 187–198
31. Coury, A. J., and Tjian, R. (1988) *Cell* **55,** 887–898
32. Suske, G. (1999) *Gene* **238,** 291–300
33. Dennig, J., Beato, M., and Suske, G. (1996) *EMBO J.* **15,** 5659–5667
34. Majello, B., De Luca, P., and Lania, L. (1997) *J. Biol. Chem.* **272,** 4021–4026
35. Ammendola, R., Mesuraca, M., Russo, T., and Cimino, F. (1992) *J. Biol. Chem.* **267,** 17944–17948
36. Saffer, J. D., Jackson, S. P., and Annarella, M. B. (1991) *Mol. Cell. Biol.* **11,** 2189–2199
37. Daniel, S., Zhang, S., Depaoli-Roach, A. A., and Kim, K.-H. (1996) *J. Biol. Chem.* **271,** 14692–14697
38. Xiao, H., Hasegawam, T., and Isobe, K. (2000) *J. Biol. Chem.* **275,** 1371–1376
39. Lee, T. H., Chuang, L. Y., and Hung, W. C. (2000) *Oncogene* **19,** 3766–3773
40. Xu, Q., Ji, Y. S., and Schmedtje, J. F., Jr. (2000) *J. Biol. Chem.* **275,** 24583–24589
41. Tretiakova, A., Steplewski, A., Johnson, E. M., Khalili, K., and Amini, S. (1999) *J. Cell. Physiol.* **181,** 169–186

GENES: STRUCTURE AND REGULATION:

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